Supporting Information for

Combining biocatalysis and organocatalysis for the synthesis of piperidine alkaloids.

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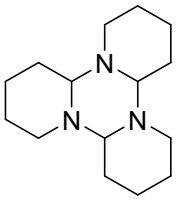
General Methods and Materials

Methods: NMR spectra were recorded on a Bruker Avance 400 or a Varian Oxford AS400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). The chemical shift values (δ) are reported in ppm with the residual solvent referenced to $CDCl_3$: δ 7.26 for ^{1}H-NMR, δ 77.0 for ^{13}C NMR; or MeOD: δ 3.31 for ^{1}H-NMR, δ 49.0 for ^{13}C NMR. Coupling constants (*J*) are reported in Hz and refer to the observed peak multiplicities. GC analysis was performed using a Nexis GC-2030 chromatograph equipped with a flame ionising detector, an AOC-20s autosampler and an achiral SHIMADZU SH-Rxi-5ms Crossbond® column (30 m x 0.25 mm x 0.25 µm). The front inlet temperature was set to 230 °C and the front detector was set to 270 °C. Split flow was set to 158.2 $mL.min^{-1}$ and the nitrogen gas was set to a constant flow of 1.92 $mL.min^{-1}$. Temperature program: 40 °C hold for 2 minutes followed by 20 $°C.min^{-1}$ temperature rise to 150 °C and then a hold for 5 minutes followed by a 30 $°C.min^{-1}$ temperature rise to 270 °C and a further hold for 10 minutes. Analytical samples were prepared for GC analysis by basifying (pH ~13) with 50 µL NaOH (10 M) and extracted with EtOAc (800 µL). Mass spectra were recorded on a Bruker MicroTOF II spectrometer using Electron Spray Ionization (ESI) or an Agilent 6546 LC/Q-TOF using ESI. IR spectra were recorded on a Bruker ATR. Chiral HPLC was performed using an CHIRALCEL OD-RH column (150 x 4.6 mm, with a particle size of 5 µm). An isocratic method was used with H_2O:AcN (52:48) at 0.3 mL/min for 40 minutes. Absorbance was measured at 254 nm with a background measurement at 360 nm.

Materials: Commercially available reagents and solvents were purchased from Acros Chemicals, Fluorochem, Sigma Aldrich and Thermo Fisher Scientific and were used without further purification. Thin layer chromatography was performed on Alfa Aesar silica gel 60 F254 plates. Flash column chromatography was performed on silica gel (60 Å, 230-400 mesh). Commercially available transaminase, ATA256, was purchased from Codexis® in the form of lyophilized cell extract.

Synthesis of α-tripiperideine[1]



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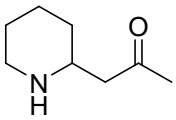
N-Chlorosuccinimide (8.23 g, 61.6 mmol) was suspended in Et_2O and cooled to 0°C before piperidine (5.0 g, 5.8 mL, 58.7 mmol) was added dropwise and the suspension was then stirred at r.t. for 2 hours. The resultant mixture was filtered and washed with Et_2O before the filtrate was washed with water (30 mL), dried over Na_2SO_4 and concentrated *in vacuo* (without heating) to give crude 1-chloropiperide as a yellow oil. Crude 1-chloropiperide was dissolved in a solution of EtOH (60 mL) and KOH (3.50 g) and the solution was refluxed for 2 h, filtered and washed with EtOH (20 mL x 3). The filtrate was concentrated *in vacuo* to ~20 mL and Et_2O (150 mL) and water were added, and the solution washed with Et_2O (3 x 20 mL). The organic fractions were combined and dried over Na_2SO_4 and concentrated *in vacuo* to give α-tripiperideine as a yellow oil (4.09 g, 84%). **^{1}H NMR** (400 MHz, $CDCl_3$) δH 3.10 - 3.03 (3H, m), 2.79 – 2.72 (3H, m), 2.02 –1.91 (3H, m), 1.74 – 1.56 (9H, m), 1.55 – 1.46 (6H, m), 1.31 – 1.15 (3H, m); **^{13}C NMR** (101 MHz, $CDCl_3$) δC 81.8 (**C**H), 46.3 (**C**H_2), 29.3 (**C**H_2), 25.9(**C**H_2), 22.2 (**C**H_2); **HRMS-ESI (m/z):** $C_{15}H_{28}N_3^+$ $[M+H]^+$ theoretical 250.2278, found 250.2278; **IR (ATR)** 3350, 2924, 2851, 1445, 1378, 1238, 1112. Data is consistent with literature.[1]

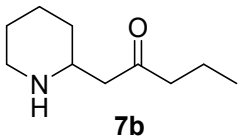
General procedure for Mannich product chemical standards

α-tripiperideine (0.82 g, 3.28 mmol), L-proline (188 mg, 1.64 mmol) and ketone (**6a-6c**) (19.7 mmol) were dissolved in acetonitrile (50 mL) before being stirred at r.t. for 48 h. The reaction mixture was diluted in DCM (70 mL) and washed with brine (3 x 40 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified *via* column chromatography and concentrated *in vacuo* to give the product as an oil.

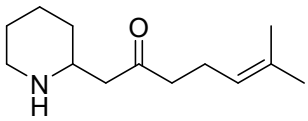
Mannich products



7a Derived from ketone **6a**. Yellow oil (209 mg, 43%) eluted in DCM/MeOH (90:10). **^{1}H NMR** (400 MHz, $CDCl_3$) δH 3.02 – 2.95 (1H, m, C**H**$_2$), 2.94 – 2.88 (1H, m, C**H**), 2.64 (1H, td, *J* = 11.9, 2.9 Hz, C**H**$_2$), 2.48 (2H, d, *J* = 6.4 Hz, C**H**$_2$), 2.12 (3H, s, C**H**$_3$), 1.88 (1H, s, broad, N**H**), 1.78 – 1.69 (1H, m, C**H**$_2$), 1.60 – 1.50 (2H, m, C**H**$_2$), 1.41– 1.30 (2H, m, C**H**$_2$), 1.17 – 1.06 (1H, m, C**H**$_2$); **^{13}C NMR** (100 MHz, $CDCl_3$) δC 208.4 (**C**=O), 52.3 (**C**H), 50.8 (**C**H$_2$), 46.8 (**C**H$_2$), 32.5 (**C**H$_2$), 30.6 (**C**H$_3$), 26.0 (**C**H$_2$), 24.6 (**C**H$_2$); **HRMS-ESI (m/z):** $C_8H_{16}NO^+$ $[M+H]^+$ theoretical 142.1226, found 142.1228; **IR (ATR)** 3325, 2925, 2852, 1707, 1331, 1165, 1077. Data consistent with literature.[1]



7b Derived from ketone **6b**. Yellow oil (222 mg, 40%) eluted in DCM/MeOH (95:5). **^{1}H NMR** (400 MHz, $CDCl_3$) δH 3.01 – 2.94 (1H, m, C**H**$_2$), 2.94-2.87 (1H, m, C**H**), 2.65 – 2.56 (m, 1H, C**H**$_2$), 2.48 – 2.40 (2H, m, C**H**$_2$), 2.30 (2H, t, *J* = 7.3 Hz, C**H**$_2$), 1.73 – 1.65 (1H, m, C**H**$_2$), 1.57 – 1.46 (4H, m, C**H**$_2$), 1.44 – 1.24 (2H, m, C**H**$_2$), 1.19 – 1.10 (1H, m, C**H**$_2$), 0.84 (3H, t, *J* = 7.4 Hz,C**H**$_3$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 210.7 (**C**=O), 52.5 (**C**H), 49.4 (**C**H$_2$), 46.7 (**C**H$_2$), 45.4 (**C**H$_2$), 32.2 (**C**H$_2$), 25.6 (**C**H$_2$), 24.4 (**C**H$_2$), 17.0 (**C**H$_2$), 13.7 (**C**H$_3$); **HRMS-ESI (m/z):** $C_{10}H_{20}NO^+$ 170.1545 $[M+H]^+$, found 170.1542. Data is consistent with literature.[2]



7c Derived from ketone **6c**. Brown oil (507 mg, 74%) eluted in DCM/MeOH/Et_3N (95:5:0.1). **^{1}H NMR** (400 MHz, $CDCl_3$) δH 5.06 – 5.00 (1H, m, C**H**), 3.02 – 2.95 (1H, m, C**H**$_2$), 2.95 – 2.88 (1H, m, C**H**), 2.64 (1H, td, *J* = 11.7, 2.8 Hz, C**H**$_2$), 2.47 -2.38 (3H, m, C**H**$_2$), 2.27 – 2.19 (2H, m, C**H**$_2$), 1.78 – 1.72 (1H, m, C**H**$_2$), 1.67 – 1.65 (3H, s, C**H**$_3$), 1.62 – 1.58 (3H, s, C**H**$_3$),1.57 – 1.51 (2H, m, C**H**$_2$), 1.45 – 1.26 (3H, m, C**H**$_2$), 1.19 – 1.07 (1H, m, C**H**$_2$); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 210.4 (**C**=O),

132.7 (CH=**C**(CH3)$_2$), 122.6 (**C**H), 52.3 (**C**H), 49.9 (**C**H$_2$), 46.7 (**C**H$_2$), 43.4 (**C**H$_2$), 32.5 (**C**H$_2$), 26.0 (**C**H$_2$), 25.6 (**C**H$_3$), 24.7 (**C**H$_2$), 22.4 (**C**H$_2$), 17.6 (**C**H$_3$); **HRMS-ESI (m/z):** $C_{13}H_{24}NO^+$ 210.1858 $[M+H]^+$, found 210.1853; **IR (ATR)** 3318, 2925, 2853, 1707, 1638, 1440, 1376, 1287, 1121.

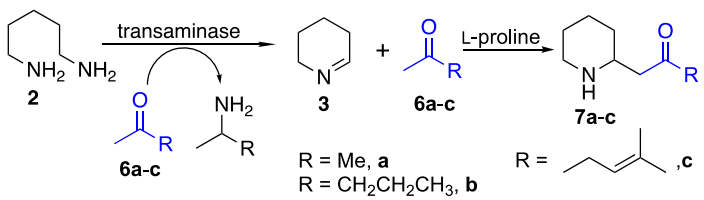
Mannich reaction under biotransformation conditions

A solution of α-tripiperideine (3.33 mM, 1 mL) in HEPES buffer (100 mM, pH 10), containing L-proline (0, 2, 10, 20, 50, 70 or 100 mM) and ketone (**6a-c**) (200 mM) in 10% DMSO was prepared. The microfuge tubes were then incubated at 37°C, 200 rpm, for 24 h. The reaction was basified with NaOH (50 µL) and ethyl acetate (800 µL) was added, the mixture was centrifuged (13000 rpm, 2 min) and the organic layer was analysed by GC-FID.

Analytical scale biotransformations towards Mannich products

A 500 µL solution of cadaverine dihydrochloride (10, 50, 100 or 150 mM) in HEPES buffer (100 mM, pH 10, PLP (1 mM)) containing L-proline (0, 10, 20, 50, 70 or 100 mM) and ketone (**6a-c**) (200 mM) in 10% DMSO was prepared. Solution is pH adjusted to pH 10. The final volume was adjusted to 1 mL with an enzyme solution containing the commercially available ATA256 (10 mg/mL), HEPES buffer (100 mM, pH 10, PLP (1mM)) and 10% DMSO. The mixture was incubated at 200 rpm, 37 or 50 °C for 24 or 48 h. The reaction was basified with NaOH (50 µL) and ethyl acetate (800 µL) was added, the mixture was centrifuged (13000 rpm, 2 min) and the organic layer was analysed by GC-FID.

Table S1. Temperature and time optimization of L-proline-catalysed Mannich reaction with ketones **6a-c**.



Entry	Ketone	Temp (°C)	Time (h)	Conv. (%)	Product
1	**6a**	37	24	73	**7a**
2	**6a**	37	48	75	**7a**
3	**6a**	37	24	68	**7a**
4	**6a**	37	48	71	**7a**
5	**6b**	37	24	21	**7b**
6	**6b**	37	48	30	**7b**
7	**6b**	50	24	23	**7b**
8	**6b**	50	48	23	**7b**
9	**6c**	37	24	11	**7c**
10	**6c**	37	48	12	**7c**
11	**6c**	50	24	10	**7c**
12	**6c**	50	48	10	**7c**

Conditions: Cadaverine **2** (10 mM), ketone **6a**-**c** (200 mM), ATA256 (5 mg mL), L-proline (100 mM), HEPES (100 mM, pH 10), PLP (1 mM), DMSO (10% v/v), 37 or 50 °C, 200 rpm, 24 or 48 hours. Conversion was measured by GC-FID and values represent the mean of least three replicates.

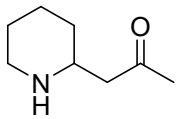
Table S2. Amine donor optimization screen of L-proline-catalysed Mannich reaction with ketones **6a**-**c**.

Entry	Ketone	Substrate conc. (mM)	Conv. (%)	Product Conc. (mM)
1	**6a**	10	75	8
2	**6a**	50	65	32

3	**6a**	100	37	37
5	**6b**	10	30	3
6	**6b**	50	20	10
7	**6b**	100	11	11
9	**6c**	10	12	1
10	**6c**	50	5	3
11	**6c**	100	3	3

Conditions: Cadaverine **2** (10, 50 or 100 mM), ketone **6a**-**c** (200 mM), ATA256 (5 mg mL), L-proline (100 mM), HEPES (100 mM, pH 10), PLP (1 mM), DMSO (10% v/v), 37 ℃, 200 rpm, 24 or 48 hours. Conversion was measured by GC-FID and values represent the mean of least three replicates.

Preparative scale biocatalytic synthesis of 7a



7a

A 20 mL solution of cadaverine dihydrochloride (50 mM) in HEPES buffer (100 mM, PLP (1 mM)) containing acetone (200 mM), L- or D-proline (100 mM) and ATA256 (5 mg/mL) in 10% DMSO was prepared. The solution was incubated at 37°C, 200 rpm for 48 h. This solution was basified with NaOH (pH 12.0) and washed with Et_2O (3 x 20 mL). The combined organic extracts were dried over MgSO4 and concentrated *in vacuo*. The resultant oil was purified *via* column chromatography (90:10/DCM:MeOH) to afford **7a** as a light-yellow oil; L-Proline (80 mg, 57%), D-proline (85 mg, 60%). *See general procedure for the synthesis of the chemical standard for the full characterisation of **7a**.*

GC traces and standard curves

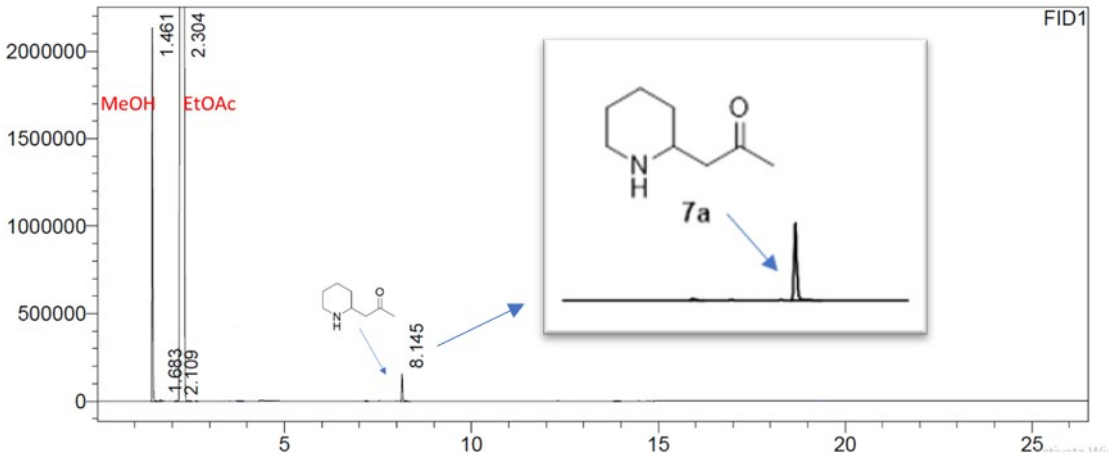


Figure S1a - GC trace of chemically synthesised **7a** (25 mM in EtOAc). See general procedures for synthesis.

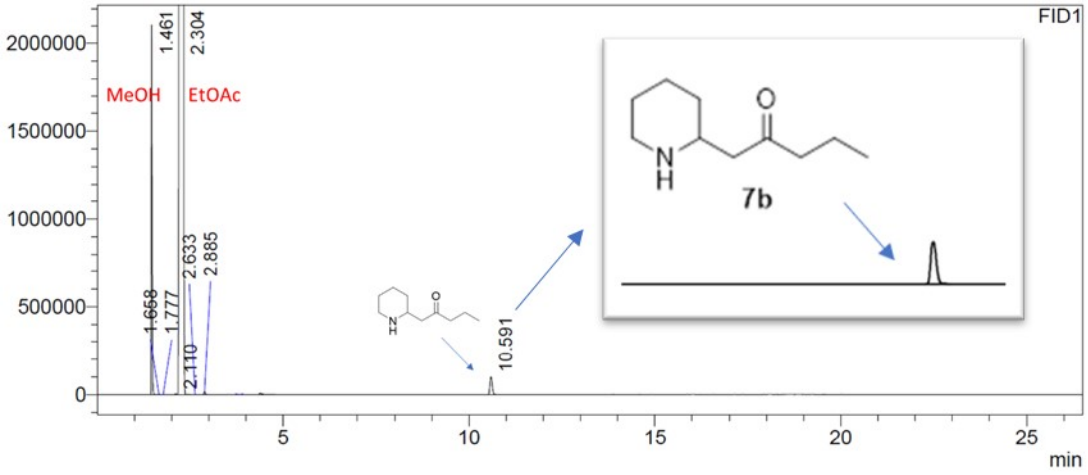


Figure S2a - GC trace of chemically synthesised **7b** (25 mM in EtOAc). See general procedures for synthesis.

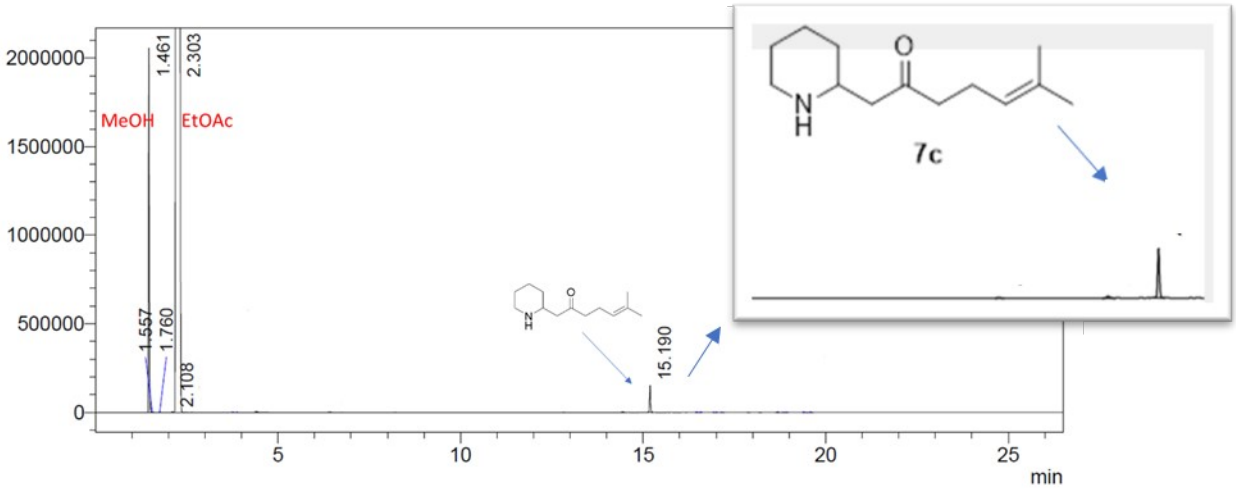


Figure S3a - GC trace of chemically synthesised **7c** (25 mM in EtOAc). See general procedures for synthesis.

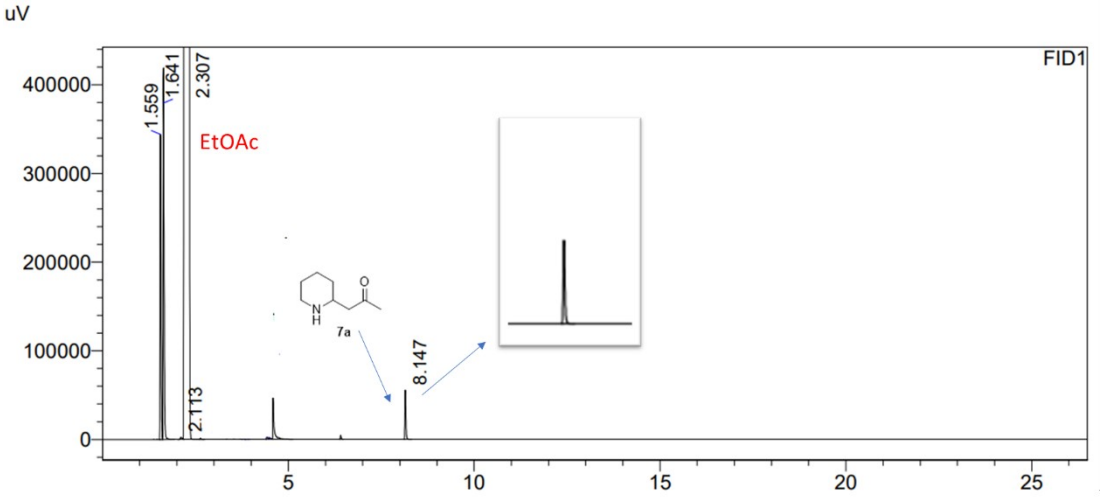


Figure S1b - GC trace of biotransformation to synthesise **7a**, using cadaverine (10 mM), acetone (200 mM), L-proline (100 mM), ATA-256 (5 mg mL^{-1}), HEPES (100 mM, PLP (1 mM)), DMSO (10 % v/v), 37°C, 48 h, 200 rpm. 77% conversion to product **7a** was achieved. **Note**: higher concentrations of L-proline caused larger inconsistencies in the % conversion of **6a** to **7a** (values ranged from 72-88% for Table 1 entry 9 and 71-77 % for Table 2 entry 1) via GC analysis. The extraction step is suspected to be the cause of this variability.

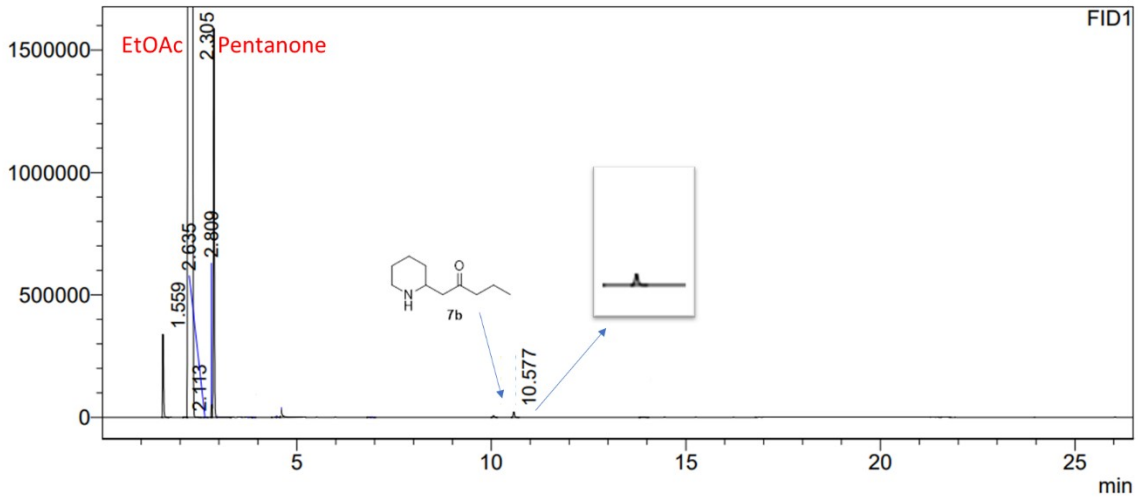


Figure S2b - GC trace of biotransformation to make **7b**, using cadaverine (10 mM), pentanone (200 mM), L-proline (100 mM), ATA-256 (5 mg mL-1), HEPES (100 mM, PLP (1 mM)), DMSO (10 % v/v), 37°C, 48 h, 200 rpm. 30% conversion to product **7b** was achieved.

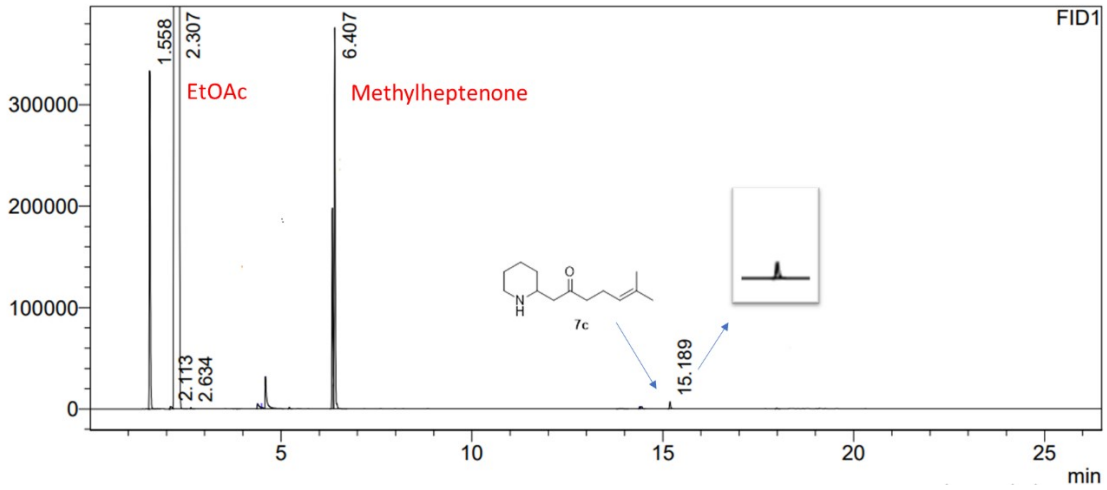


Figure S3b - GC trace of biotransformation to make **7c**, using cadaverine (10 mM), methyheptenone (200 mM), L-proline (100 mM), ATA-256 (5 mg mL-1), HEPES (100 mM, PLP (1 mM)), DMSO (10 % v/v), 37°C, 48 h, 200 rpm. 12% conversion to product **7c** was achieved.

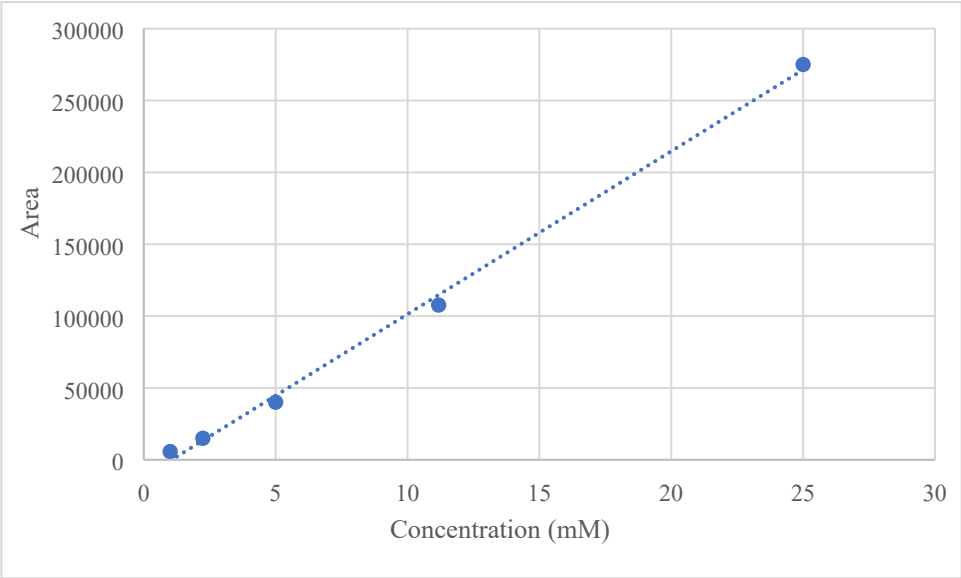


Figure S4 - Concentration of **7a** (mM) vs peak area. Standard curve to calculate conversion to **7a.**

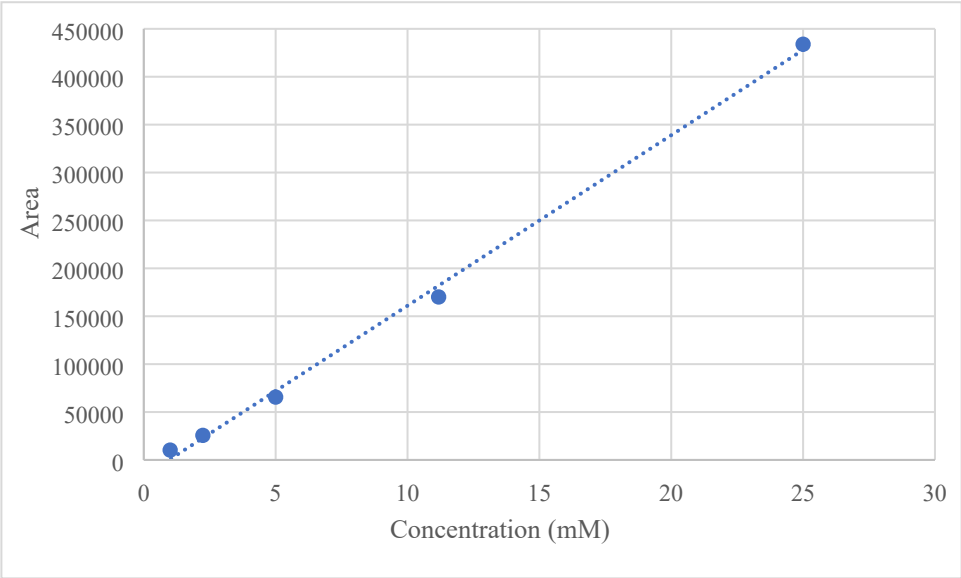


Figure S5 - Concentration of **7b** (mM) vs peak area. Standard curve to calculate conversion to **7b**.

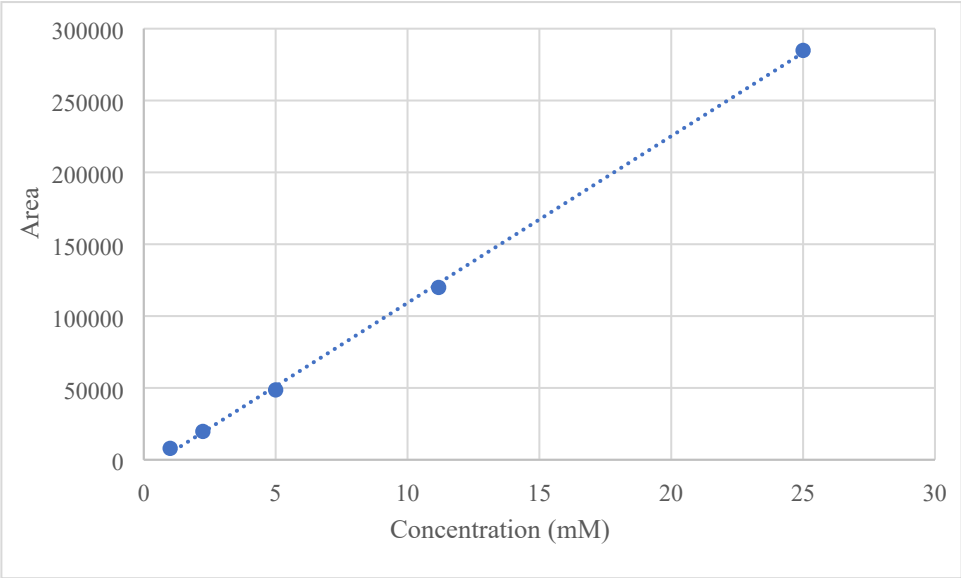


Figure S6 - Concentration of **7c** (mM) vs peak area. Standard curve to calculate conversion to **7c**.

Chiral HPLC traces

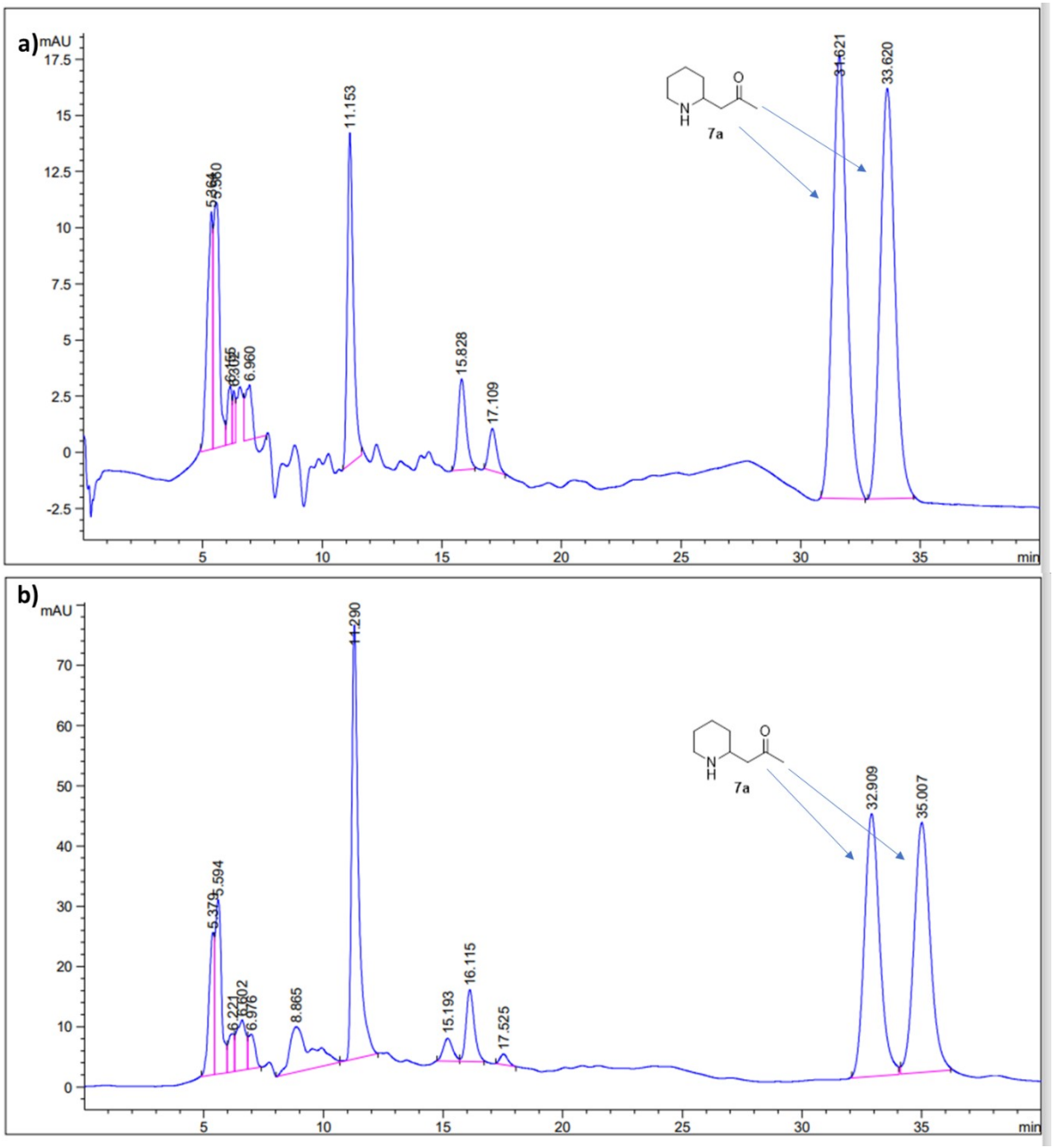
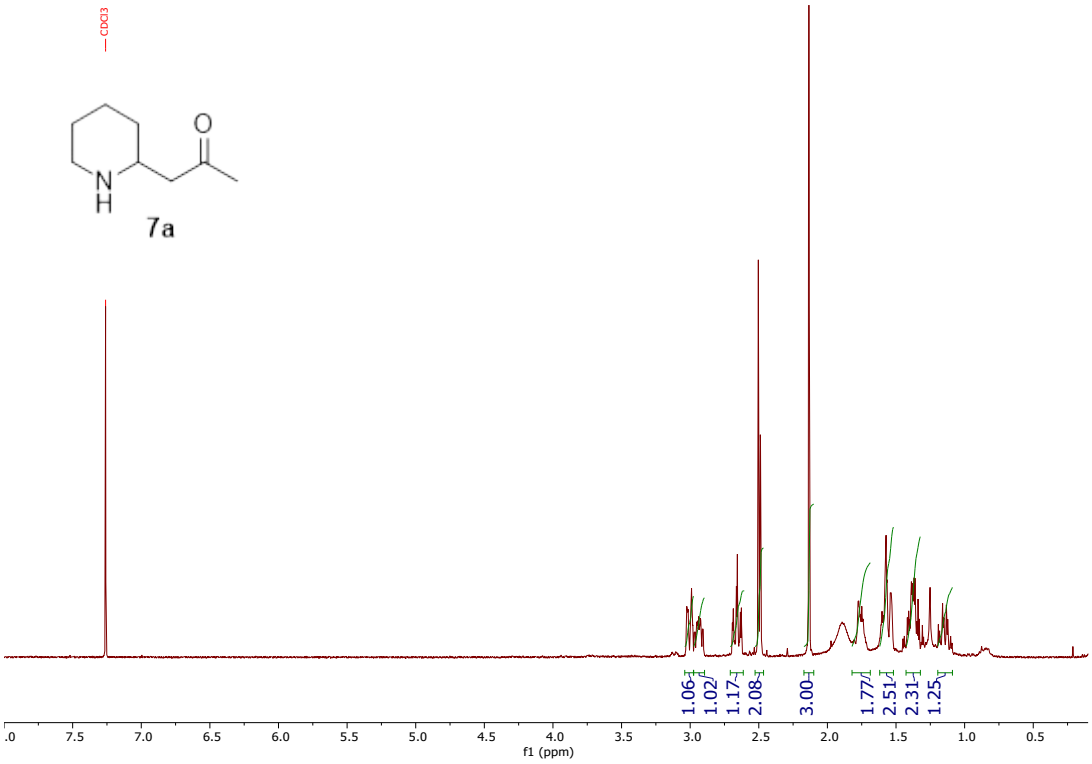
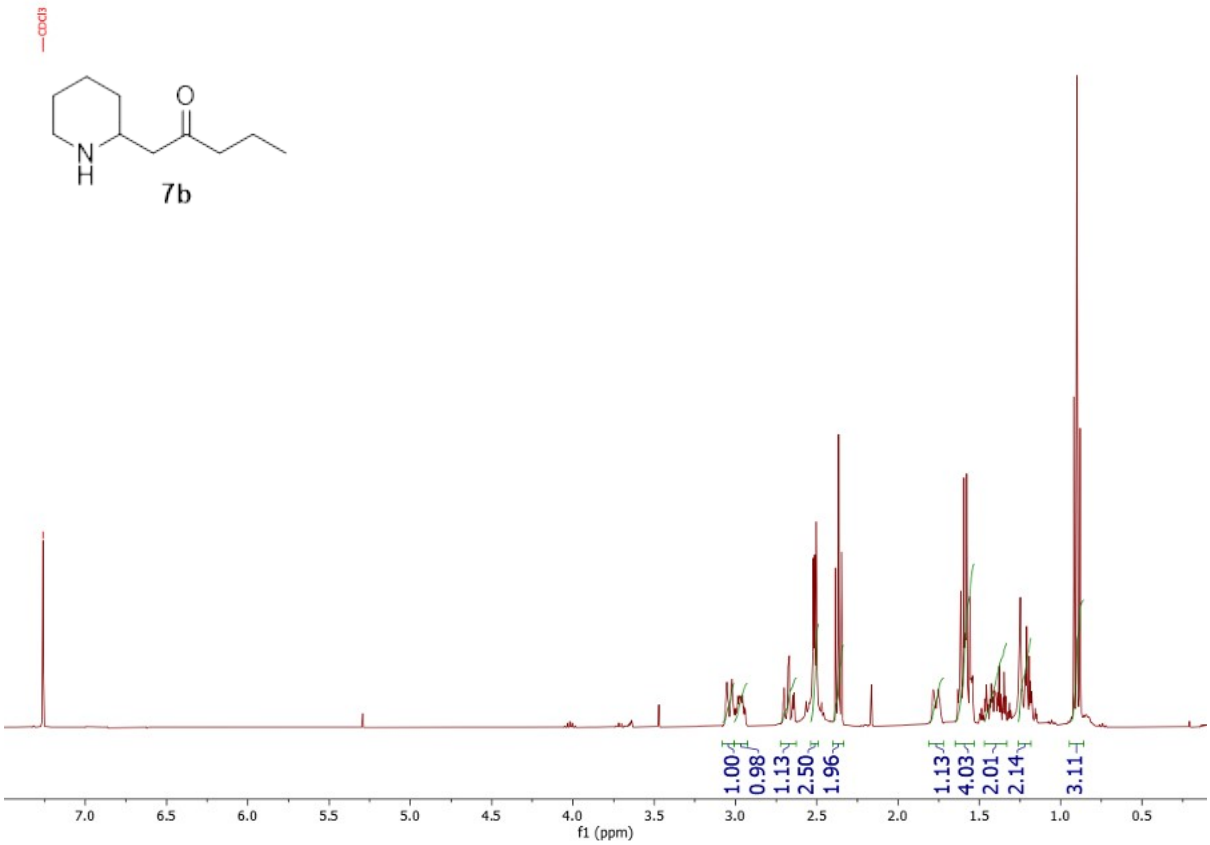


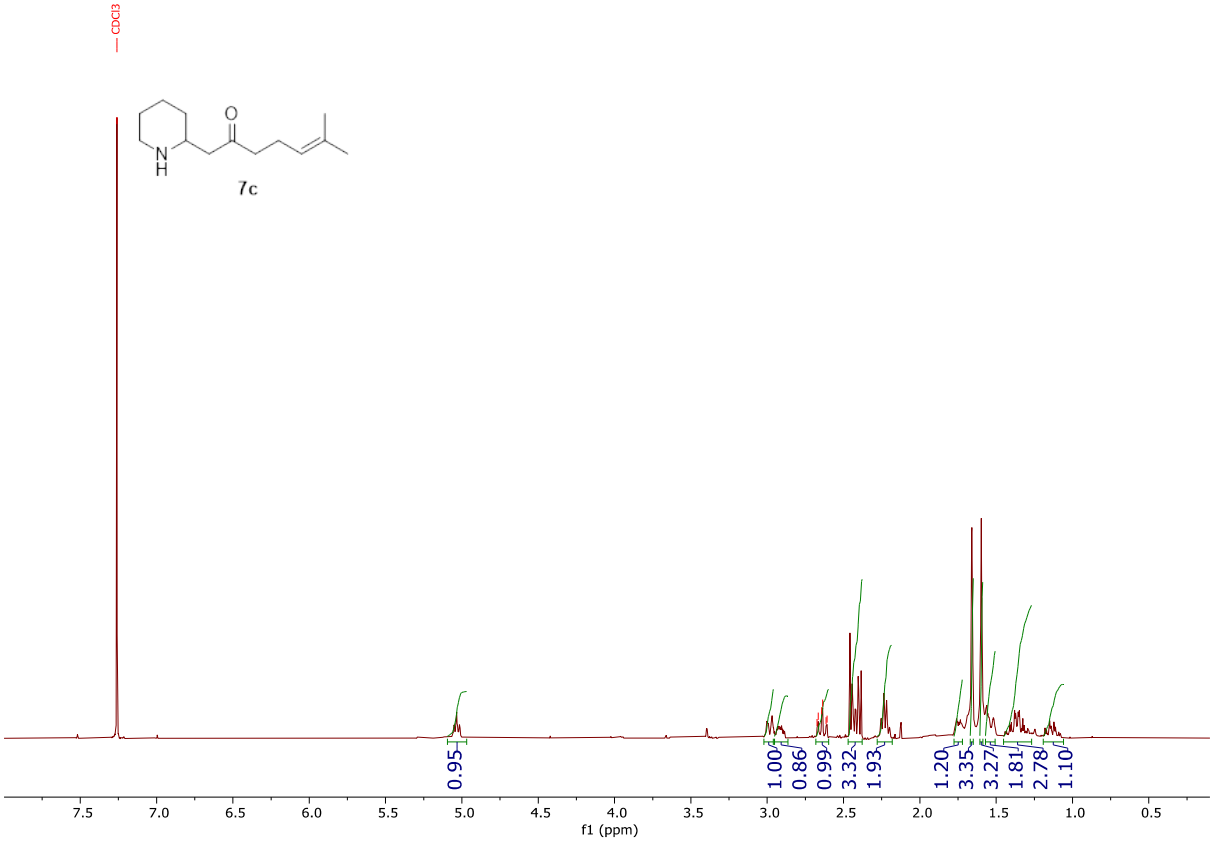
Figure S7 – Chiral HPLC traces for **7a** produced chemically (**a**) and biocatalytically (**b**), showing the product is racemic. See general methods for HPLC protocol. Impurities in the spectra do not arise from the compound, and are likely from glassware or the HPLC column. The NMR of the biotransformation (see below) shows the product is clean

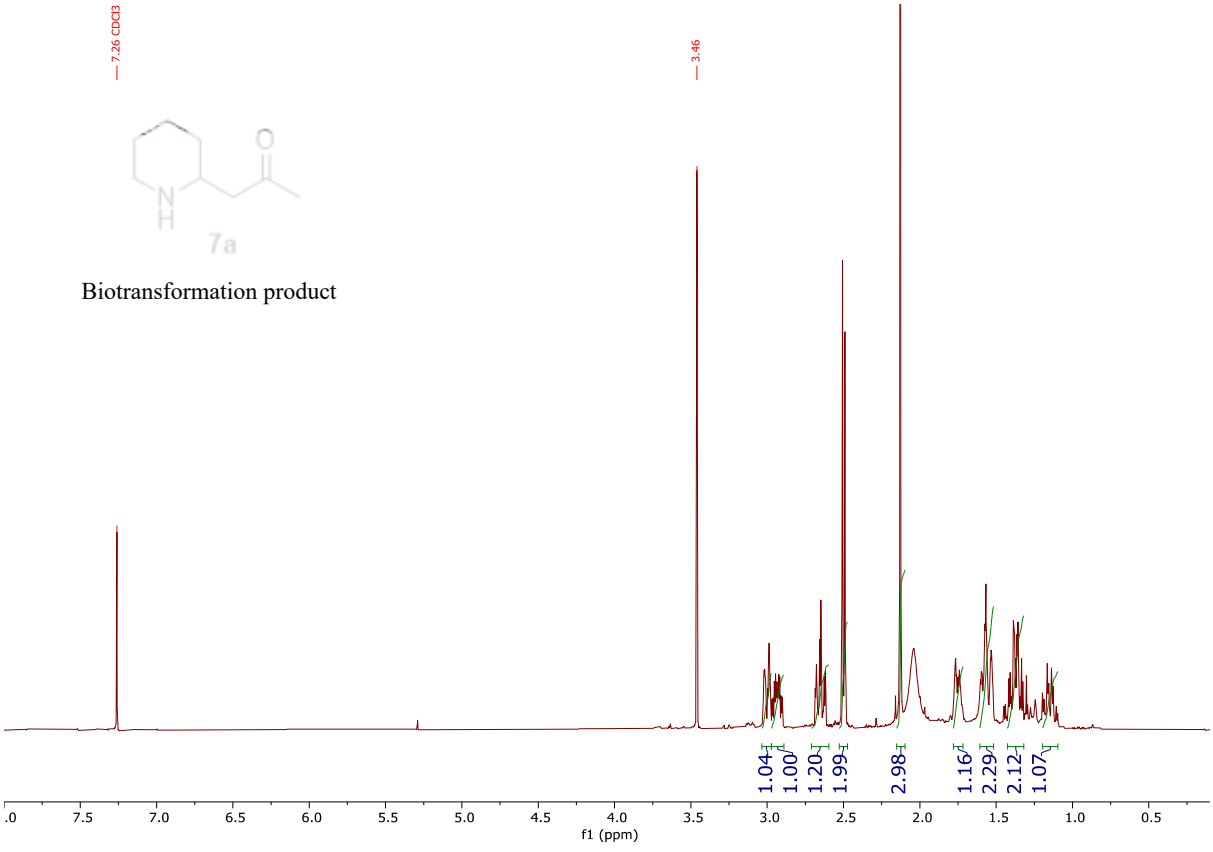
NMR Data

NMR spectra is of chemical standards, unless otherwise stated.



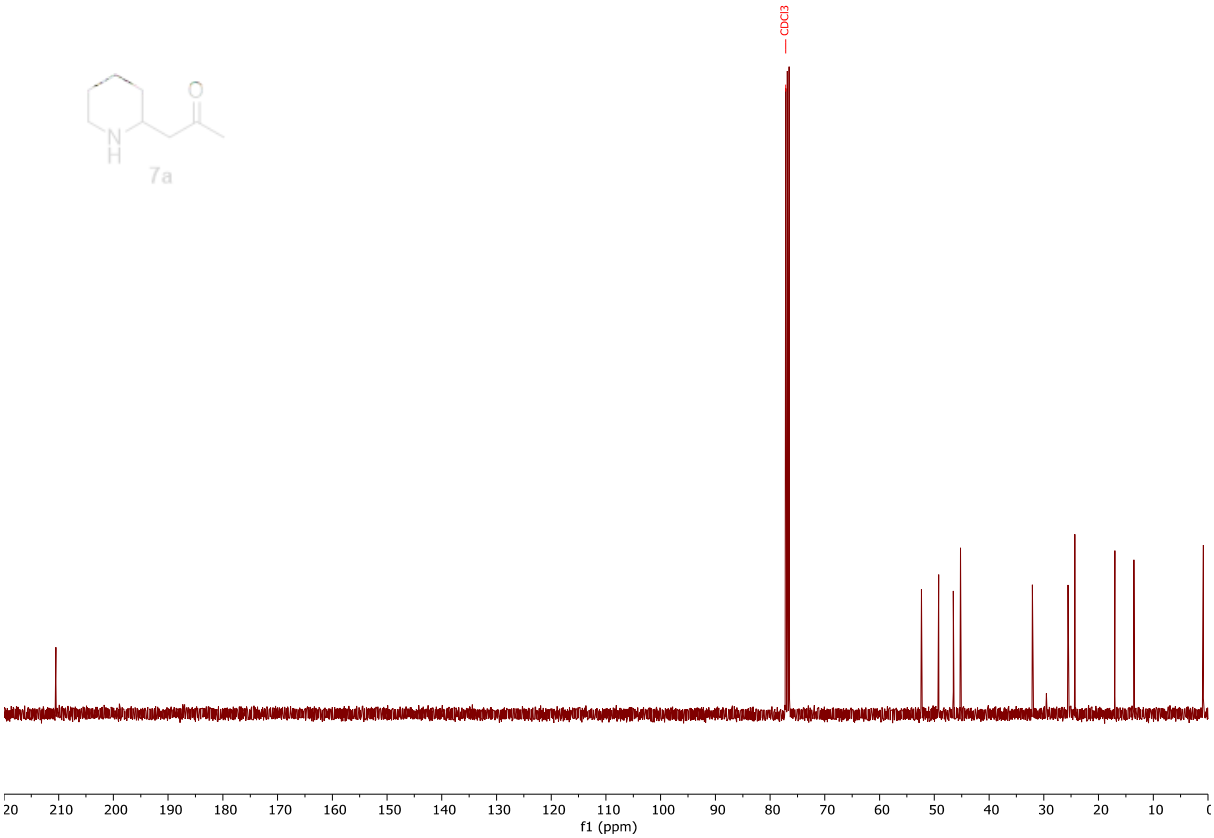


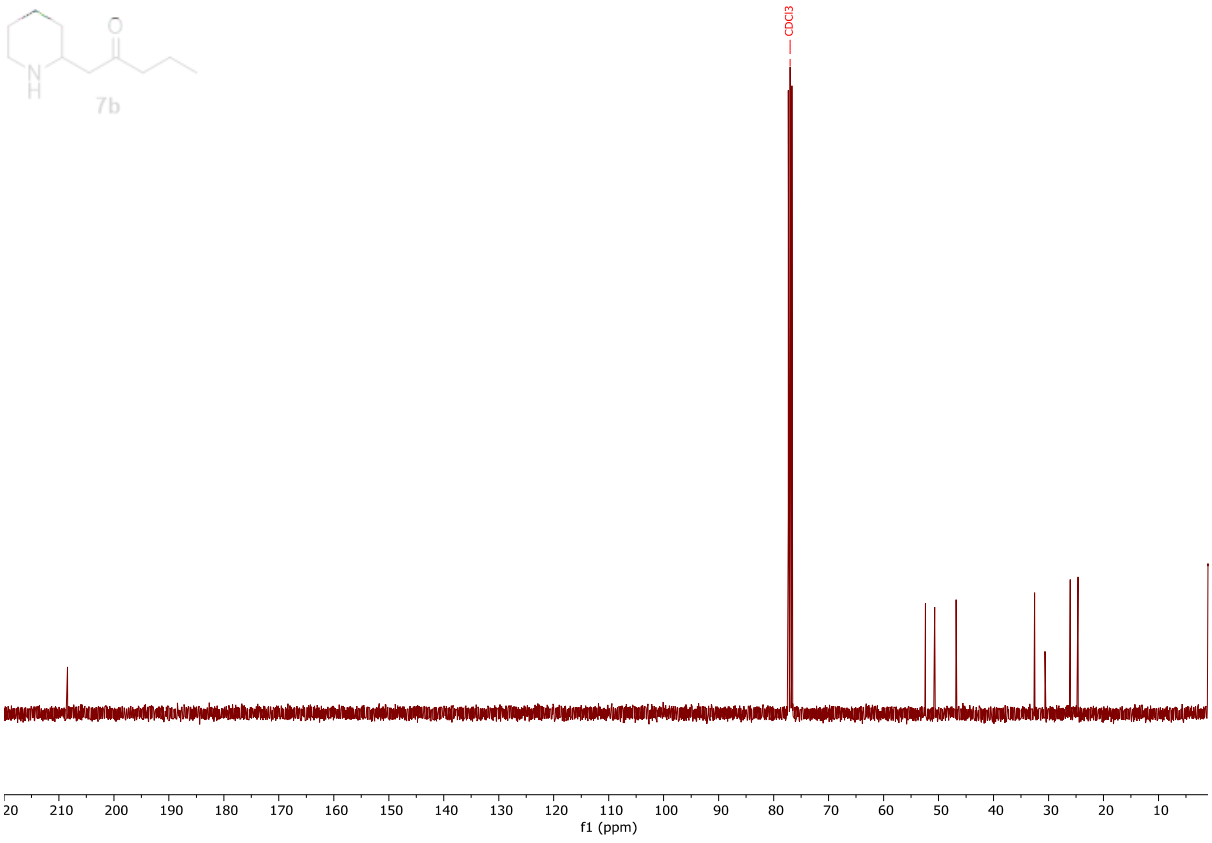


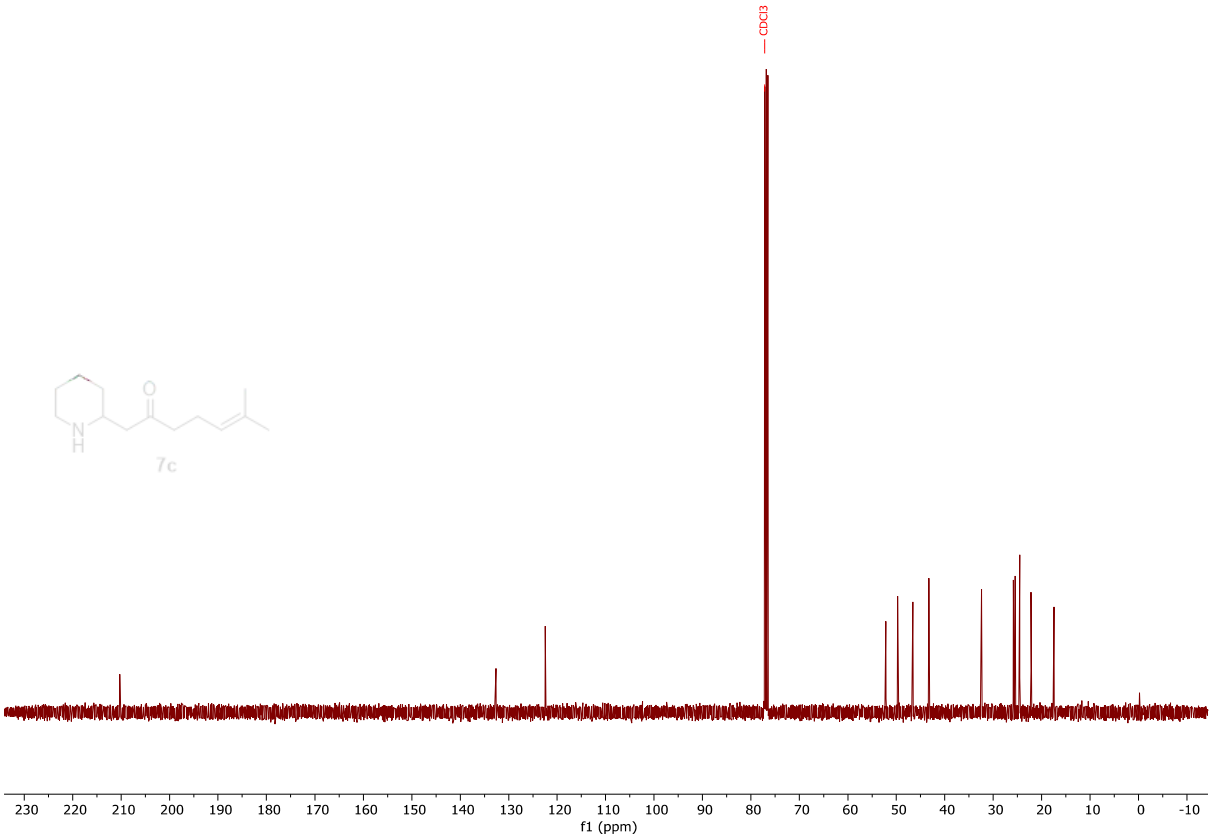


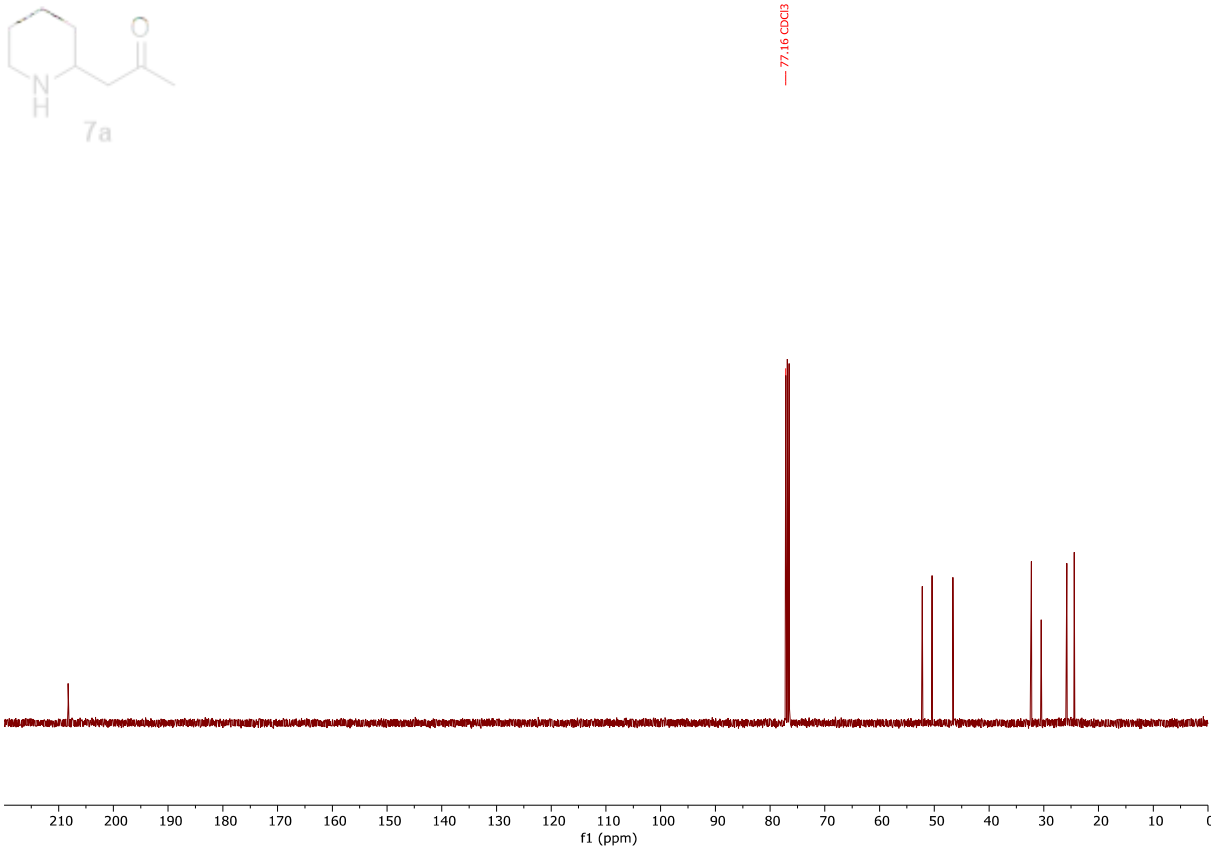
Biotransformation product

NMR spectra of biotransformation product **7a**.






CDCl3
O
N
H
7c
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


77.16 CDCl3
O
N
H
7a
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

NMR spectra of the biotransformation to make **7a**.

References

1 M. R. Monaco, P. Renzi, D. M. Scarpino Schietroma and M. Bella, *Organic Letters*, 2011, **13**, 4546–4549.

2 J. L. Galman, I. Slabu, F. Parmeggiani and N. J. Turner, *Chemical Communications*, 2018, **54**, 11316–11319.